PATENT
CASE:JB0600Q

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	:	
RAVNIKAR *et al.*	:	Examiner: RAILEY, J.
For Patent For:	:	Group Art Unit: 1636
EXPRESSION OF SOLUBLE HETEROLOGOUS PROTEINS IN BACTERIA UTILIZING A THIOREDOXIN/PROTEIN EXPRESSION VECTOR	:	
Serial No.:08/846,606	:	
Filed: April 30, 1997	:	

Schering-Plough Corporation
Kenilworth, New Jersey 07033

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.131

Sir:

We, Paula D. Ravnikar and Robert Greenberg, declare as follows:

1. That we are the co-inventors of the subject matter disclosed and claimed in the above-identified application;

2. That we are employed by the Schering-Plough Research Institute (SPRI) which is a division of Schering Corporation, the assignee of the above-identified application;

2. That we caused experiments to be carried out in the United States of America which resulted, prior to October 1, 1995, in the construction of a vector containing both a nucleic acid sequence encoding a thioredoxin protein and a nucleic acid encoding a heterologous protein, which vector was capable of causing the expression of the thioredoxin

protein and the heterologous protein as separate, non-fused proteins wherein the heterologous protein was expressed in soluble form;

3. That Exhibits A-C attached to this Declaration are true copies of pages from a permanently bound Notebook numbered 31163, assigned to Paula Ravnikar and maintained at SPRI ("the Ravnikar Notebook");

4. That Exhibit A consists of true copies of pages 20-27 of the Ravnikar Note Book and describes experiments resulting in the cloning of the *E. coli* thioredoxin gene;

5. That Exhibit B consists of true copies of pages 84-89 of the Ravnikar Note Book and describes experiments resulting in the translational coupling, in a single plasmid, of the *E.* coli thioredoxin gene and the human interleukin-13 gene;

6. That Exhibit C, consists of true copies of pages 92-94 and 96-98 of the Ravnikar Note Book and describes experiments demonstrating the expression of human interleukin-13 in bacteria using the coupled translational plasmid the construction of which plasmid is referred to in Exhibit B; and

7. That although the dates on the note book pages referred to in Paragraphs 3-6 have been masked, we hereby confirm that the studies described in those notebook pages were carried out in the United States of America prior to October 1, 1995.

We hereby further declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true, and further, that we make these statements with the

knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Aug 13, 98

Paula D. Ravnikar, Ph.D

Date: Aug 13, 98

Robert Greenberg, Ph.D.

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE U.S. POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON D.C. 20231 ON

August 14, 1998

DATE OF DEPOSIT

IMMAC J. THAMPOE

REGISTERED REPRESENTATIVE

8/14/98

SIGNATURE AND DATE OF SIGNATURE

Exhibit A

298-5388

20

Thioredoxin : PCR from the coli genome.

Prep coli genomic DNA from a single colony of MM 294 using Biorad instagene = according to Bio rad's instructions.

Used PCR reactions Run 5 ul on gel.

Forward primer for trxA gene with NdeI cloning site

trxa.5oli Length: 30 11:13 Type: N Check: 3551 ..

1 CCTGTGGACT TACATATGAG CGATAAAATT

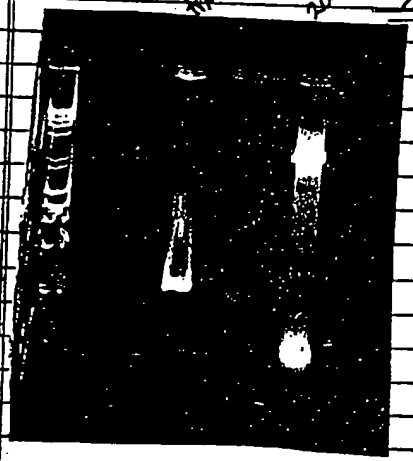
reverse primer for trxA gene with BsaBI and BamHI sites

trxa.3oli Length: 47 11:20 Type: N Check: 9729 ..

1 GCACCCAACA TCCAAGGATC CTTACCCCAG ATTAGCATCG AGGAACT

Run clean the PCR Reaction & NdeI + BamHI digest

Isolate from 1.5% gel = Glass Bean - Elute into 50 ul.



Nde. Bam Prep Vol

Ligate		Vector
Insert	15 ul	pMBS 202010
Vector	10 ul	pMBS 202020
Buffer	5 ul	
H2O	15 ul	
Ligase	1 ul	

16° overnight

Transform 294 / plate on TET Amp.

Only 202020 yielded colonies

pick 18 to screen.

No correct clones.

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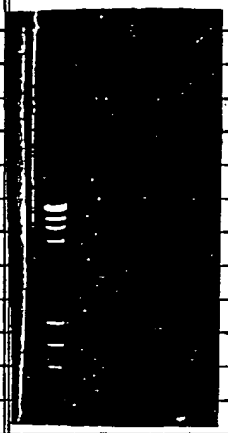
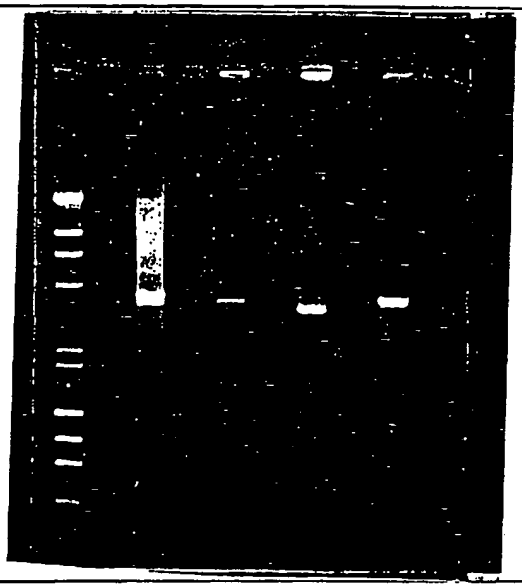
Prep 6b.

① ACYC177 2a promoter ant/Bam
② 202010 Nde/Bam
③ 202020 Ant/Nde
④ 202080 Nde/Bam
} p-trac.

Gene Clean & elute into 50ul.

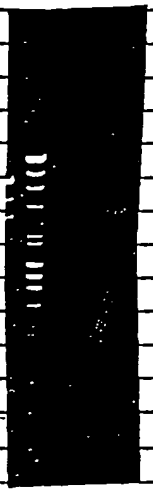
after Gene Clean.
②202020 ant/Nde
②202020 Nde/Bam
} 3ul. → ACYC177 needs to be Kinased.





ACYC177-②/Kinased
180 ul volume final.
4ul Kinase
4ul ATP (100mM stock)
37°C 30 min.
Heat inact 65°C/20 min.
EtOH ppt.
resusp 50ul.
run 3ul on Gel.

Ligation
30ul – ACYC177pro
4ul – ligase
6ul – buffer
20ul – water
–1ul before ligation
+5ul ligase



②ACYC pro②
ant Bam Kinased

② Thioredoxin
PERProduct
Nde/Bam cut
5ul of 200ul digest

Transformation
U374 + U375 / annealed.
180 pm/ul.

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This Rederin PCR Cloning

PCR Reaction : Same as from p. 20.

Nde I/ ~~Bam~~ HI digest & Prep frag on 2% agarose.
It separated into 2 bands In the headaware excised + plopped separately.

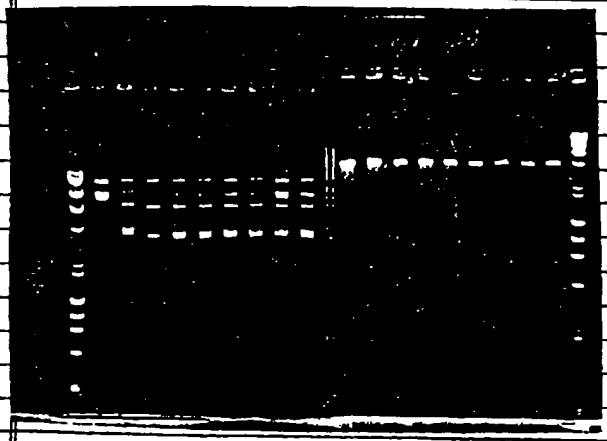
Prep Gel

Last agar gave clean

Upper & Lower bands isolated into 2 tubes

~~Trans~~ Transform 294 + select on Carb plates
The Upper frag gave larger "healthier" looking colonies + more analyzed fine
The Lower frag gave smaller + slower growing colonies

Upper #1-9 There is an Nde I Bam HI insert that has no Cla I site (gel #2)
no Xma I site (gel not shown)
Xma I/Hba I gave only a linear vector.



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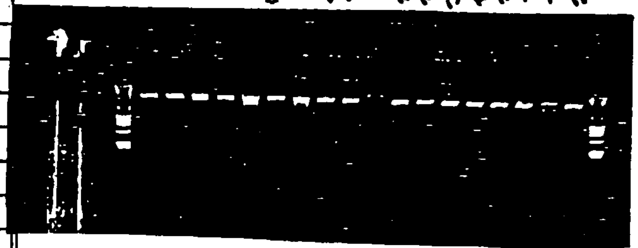
TxA lower Fragment Analysis.

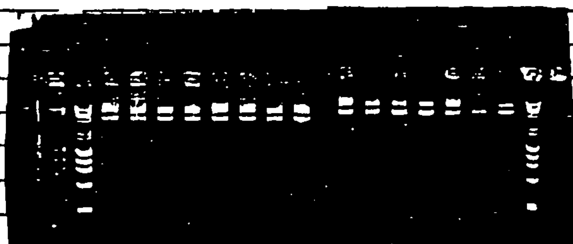
Xho/Bam.

Gel #1



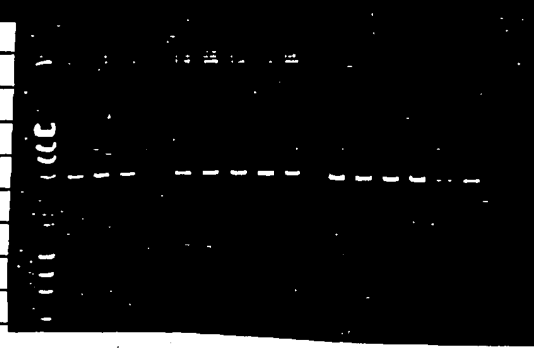
All except #5, 7, 10 have Xho Bam inserts.

Gel #2



ClaI/AatII Digest
No ClaI site in the insert
AatII vector linear.

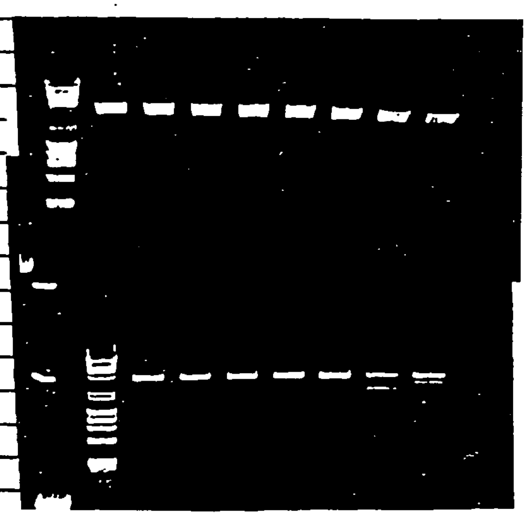
Gel #3



BsaBI Linearized #1, 2, 4, 6, 8, 9, 11, 12, 13, 14, 15, 16
#3, 17, 18 may have an extra site

BsaBI is part of the oligo used for PCR

Gel #4



XmnI/XbaI Digest

All inserts have an XmnI site.

Expect ~325 bp fragment = looks ok.

So far Everything Checks out except the absence of a ClaI site which could be a point / small variation in DNA Sequence.

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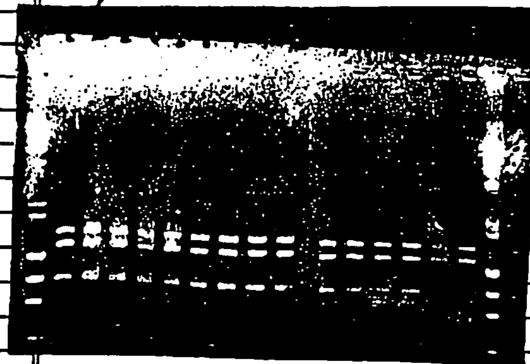
TrxA lower fragment Analysis

ApaLI.

TrxA PCR lower fragment in pMBD202020
pDR475

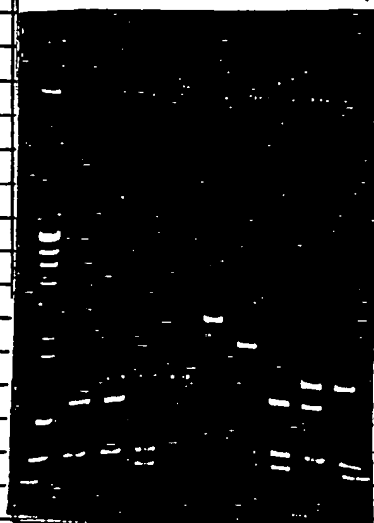


Gel [illegible]

All isolates have 3 ApaLI sites
note: #17 may have 4 sites

Need to verify ApaLI sites in the pMBD202020 vector.

Large Scale Prep of #1 & #11



λ/BstE	66
37.5 -	4360 r = -0.996
48 -	2380
51 -	2030
64 -	1350
71 -	1080

pMBD202020 vector.

④ AatII/Apa
55 - 1821
68 - 1156
71 - 1054

⑤ ApaLI
44 - 2935
68 1156

⑥ AvaI/ApaLI
48 - 2436
68 - 1156
⊕ ~500 bp.

It would appear that the ApaLI site within the lpp terminator at 401 is not present and is the result of a sequencing error or digest variation at that position.

Lanes
① Aat/ApaLI }
② /Apa } Clone #1
③ Ava/Apa }
④ Aat/Apa }
⑤ /Apa } 202020 vector
⑥ Ava/Apa }
⑦ Aat/Apa }
⑧ /Apa } Clone #11
⑨ Ava/Apa }

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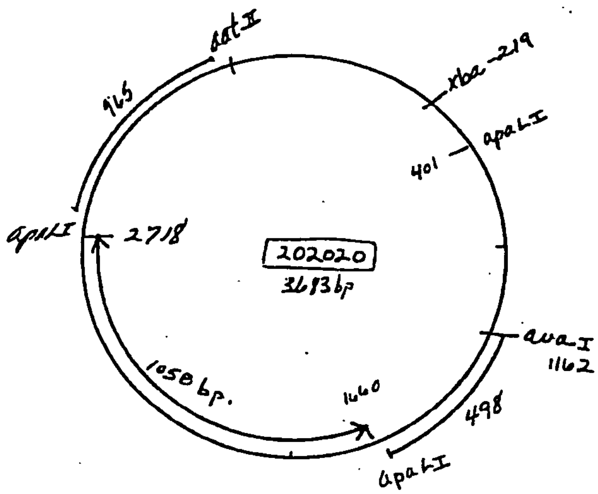
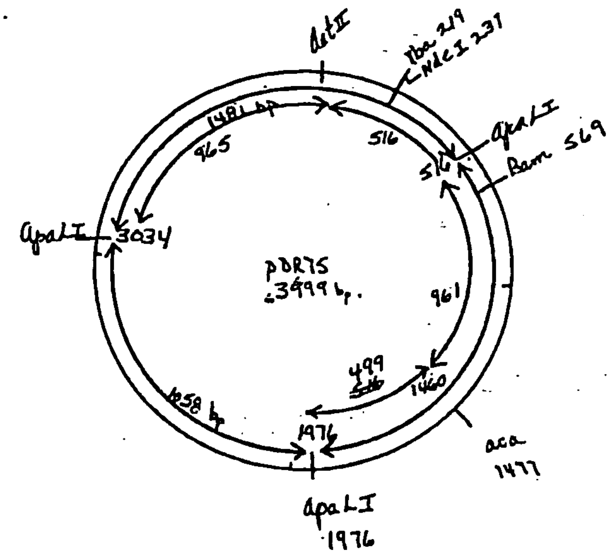
TrxA Screen Analysis.





~~3 Aat Apa~~

λ/Hd3 (6a)

r = -0.9975

20.5	4360
26	2380
28	2030
35	1350
38	1080
42	870
48	600
57.5	310

aat/Apa 1480 frag → 965 + 516

(a) largest Apa frag should be cut.

(b) 1796 → 998 + 762 ok!

insert may be a little larger than thought

ava/Apa 1460 → 961 + 499

(a) 2nd largest ~~should~~ be cut.

(b) 1272 → 998 + 572.

Clone 11

7 Aat/Apa

			Expected
32–	1272		uncut = ok!
37–	1124		uncut = ok!
39–	998	4156 total	} from 1796 = ok!
44–	762		

8 Apa

30–	1796	4192	— 1481
32–	1272		— 1460
37–	1124		1058

9 Ava/Apa

30–	1796		— uncut ok!
37–	1124		— uncut ok!
39–	998	4490	} from 1272 = ok!
50–	572		

↳ could be 516 bp

Clone 11 Appears to check out in digest better though insert could be larger than thought.

Clone I ~~digest~~ digest could be screwed up because Apa LI does not look like it did in gel #5.

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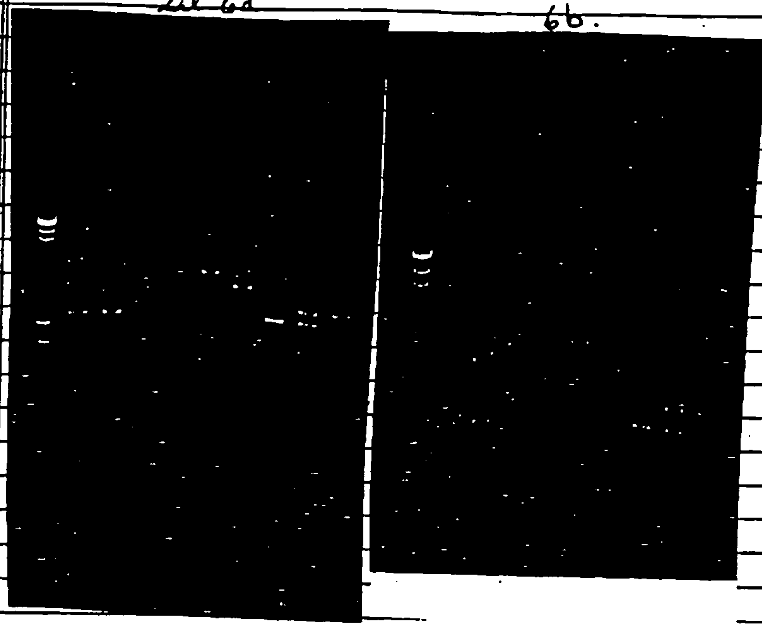
TrxA pocM cont'd.

Gel 6a 6b.



Gel 6a + 6b
Different photo exposure from p 24.

Gel #5 p.24.

λ[illegible]	
28	2326
31	2030
38	1350
42	1080
46.5	870

r = -0.9971

ApaI Digest

34	1640
36	1465
42.5	1057

#11 Sent to Seek for DNA Sequence Confirmation

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one vial of #3's 7 8 are frozen away in box.

pdr 76.

trxa-IL13 oligo
bsaBI-pshaI/bamHI

trxpsh.oli Length: 47 13:27 Type: N Check: 1217 ..

(384) 1 TAATGTGGCC GGTTCTGGTT CTGGTGATGA CGATGACAAG GGTCCGC

pshAI

Gly Ser Gly Ser Gly D D D D K. AvaII

linker enterokinase

REVERSE-COMPLEMENT of: Trxpsh.Oli check: 8911 from: 1 to: 52

trxa-IL13 oligo
bsaBI-pshaI/bamHI

trxpsh.rev Length: 51 13:28 Type: N Check: 2215 ..

(385) 1 GATCCGGGAC CCTTGTCATC GTCATCACCA GAACCAGAAC CGGCCACATT

51 A

AciI C'CGC

Cuts at:	0	6	47
Size:	6	41	

AvaII G'GnC_C

Cuts at:	0	41	47
Size:	41	6	

FauI CCCGCnnnn'nn_

Cuts at:	0	1	47
Size:	1	46	

HphI GGTGAnnnnnnn_n'

Cuts at:	0	35	47
Size:	35	12	

MspI C'CG_G

Cuts at:	0	44	47
Size:	44	3	

NlaIV GGn'nCC

Cuts at:	0	42	47
Size:	42	5	

PshAI GACnn'nnGTC

Cuts at:	0	39	47
Size:	39	8	

Sau96I G'GnC_C

Cuts at:	0	41	47
Size:	41	6	

BsaBI-pshAI/BamHI oligo.

encodes end of TrxA + linker + enterokinase cleavage site - and will be cloning sites for future coupled sequences

Anneal oligos

ligate to pDR75-11 BsaBI/BamHI cut.

Transform 2648 select on Amp plates.

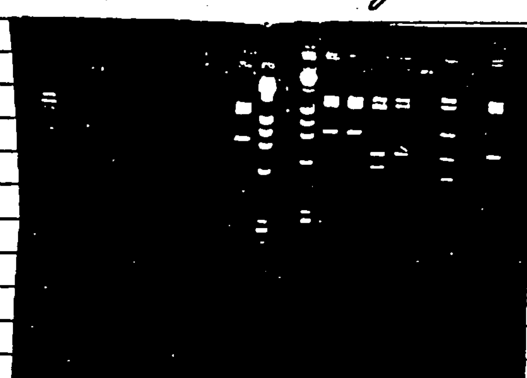
Magic Mini Screen

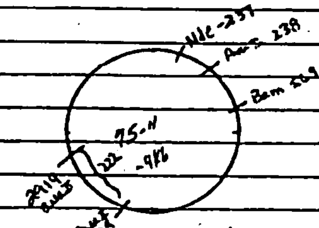
75-11 Prep Gel.

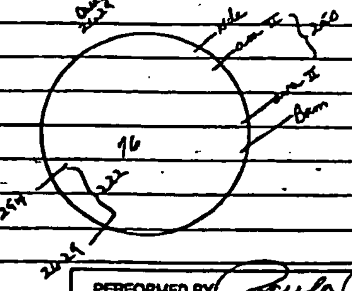
Digest with Ava II

Unfortunately a nucleotide error in the oligo destroys the BsaBI site and changes codon in the end of TrxA (leu → val)

Make a new & correct oligo.







Clones 3+7 look Correct. Digest in #7 looks Better.

Have 220/250 Doublet & ~900 bp frag. is ~650 w/ 250 bp shorter.

Note that all the Ava II sites in 2020 appear to be incompletely mapped as yet.

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Exhibit B

p.34 u471 oligo sequence used to make the trx-IL13 fusion

oligos. 467/468 pDK100
This details - IL13 coupled translation
oligo: BsaBI → SstI
Insert into pDR99/pDR88.

467/468 oligo puts an NcoI site at the ATG of IL13 for future cloning use.

3'-5' oligo for trx-il13 coupled translation

u468.oli Length: 58 11:34 Type: N Check: 7052 ..

1
>SEQED (include) of: u468.oli check: 671 from: 1 to: 68>
CACGCACAGC GGTAGACGGC GGAACGGGAC CCATGGAATA CCTCCTTAAG

51 CCAGATTT

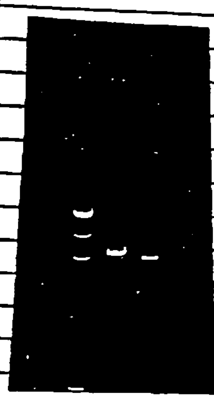
<SEQED (include) of: u468.oli check: 671 from: 1 to: 68<

coupled translation oligo for trx-il13
u467.oli Length: 62 11:30 Type: N Check: 1430 ..

1
>SEQED (include) of: trxl3cup.seq check: 4225 from: 1 to: 68>
NcoI
AAATCTGGCT TAAGGAGGTA TTCCATGGGT CCCGTTCCGC CGTCTACCGC
RBS

51 TCTGCGTGAC CT

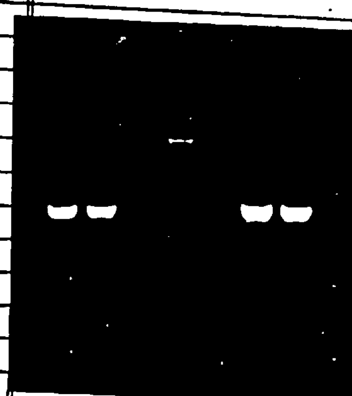
<SEQED (include) of: trxl3cup.seq check: 4225 from: 1 to: 68<



pDR88] pDR99] BsaBI

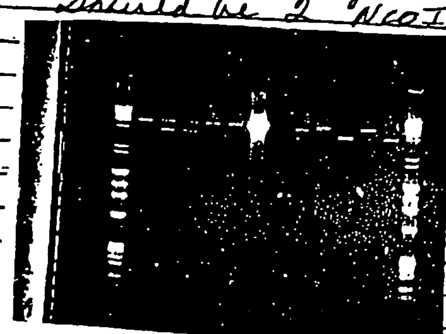
BsaBI appears to be unique in both plasmids.

annealed Oligos on a 4% NuSieve Gel
(1) 467/468 58mer
(2) 469/470 47mer
(3) 471/472 50mer
(4) 100 bp ladder



pDR88 Prep Gel.
BsaBI/SstI
PshAI/SstI

Screen NcoI digest
Should be 2 NcoI Sites



No positive clones

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Construction of pDR100 & pDR101 by annealing oligos & ligation has not proceeded well. Alternative: PCR modify IL13.
forward oligos: U 478 & U 479
Reverse oligos: U 361 & U 406 : both are downstream of BamHI site of an IL13 clone.
Template: pDR88
Clone PCR products as BsaBI/BamHI fragments into pDR88.

trxA-hIL13 translational coupling
PCR primer BsaBI cloning site
RBS overlaps the TAA stop codon
NcoI site introduced at the ATG — *Similar to U467*
U478.oli Length: 71 09:28 Type: N Check: 8193 ..

1 TTGAAAGAGT TCCTCGATGC TAATCTGGCG TAAGGAGGTA TTCCATGGGT

51 CCGGTTCCGC CGTCTACCCC T

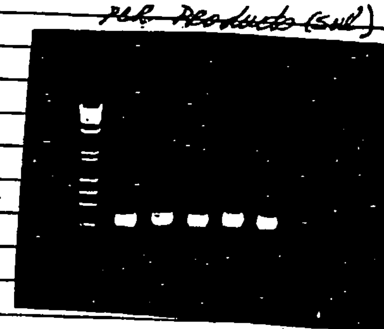
trxA-hIL13 translational coupling
PCR primer BsaBI cloning site — *Similar to U469.*
PsbAI site removed//ApaI site added atG GGC CCg

u479.oli Length: 72 09:34 Type: N Check: 9076 ..

1 TTGAAAGAGT TCCTCGATGC TAATCTGGCG TTGCAGGATG ATTAAATGGC

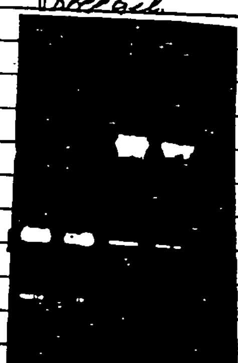
51 CCCGGTTCCG CCGTCTACCG CT

~~PCR products~~ (5 ul)



1 - λX
2 - 361+406
3 - 361 } +478
4 - 406
5 - 361 } +479
6 - 406

prep Gel.



1 361/478 } BsaBI
2 361/479
3+4 pDR88 } BamHI.

Ligation
Vector - 10
insert - 10
Buffer - 2.5
Ligase - 2.5
25 ul.

Transform w/ 294
Select on Camp plates

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Thioredoxin - hIL13 Coupled translation oligo 469/470 1"

Trx-IL13 fused plasmid has a sequence:
asp asp asp lys gly pro pro pro
GAC·GAT·GAC·AAG·GGT·CCG·GTT·CCG·CCG·etc
GAC NNNNNGTC
pshAI

Make a pshAI-SstI oligo to couple translation

asp asp asp S.D.
GAC·GAT·GAC·AA|C·GAG·GAT·GAT·TAA·ATG·GGT·CCG·etc
asn glu asp asp * met gly pro

This arrangement differs from 467/468 in the following
1) Shine Dalgarno is within a translated region
2) TAA·ATG stop & start are immediately adjacent.
3) Coupling utilizes a trx protein very similar to the one used in the fu

REVERSE-COMPLEMENT of: U470.Oli check: 4070 from: 1 to: 51

REVERSE-COMPLEMENT of: U369.Oli check: 6198 from: 1 to: 51
coupled translation oligo
pshai-ssti linker

U469.Oli Length: 51 10:24 Type: N Check: 6198 ..

1 CGAGGATGAT TAAATGGGTC CGGTTCGGCC GTCTACCGCT CTGGGTGACC

51 T

REVERSE-COMPLEMENT of: U369.Oli check: 6198 from: 1 to: 51

coupled translation oligo
pshai-ssti linker

u470.oli Length: 47 10:26 Type: N Check: 142 ..

1 CACCCAGAGC GGTAGACGGC CGAACCGGAC CCATTTAATC ATCCTCG

Tried to dig a small Xma I / SstI fragment.
There did not appear to be any positive clones.

Repeated ligation: screen with Xba·Bam & look for 930 bp fragment.

Clone # 26-29-30-34-36 looked positive
Screen done by John C. Book # 32780 p 66.

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DATE

[illegible] combinations for plasmid screening

#26-29-30-34-36. [illegible] inoculated 25 ml IFN [illegible]
Grow @ 30°C for 7hrs. 5 ml removed to [illegible]
to make [illegible] cultures.
Remainder added IPTG (100 nM) Grow ON @ 42°C.

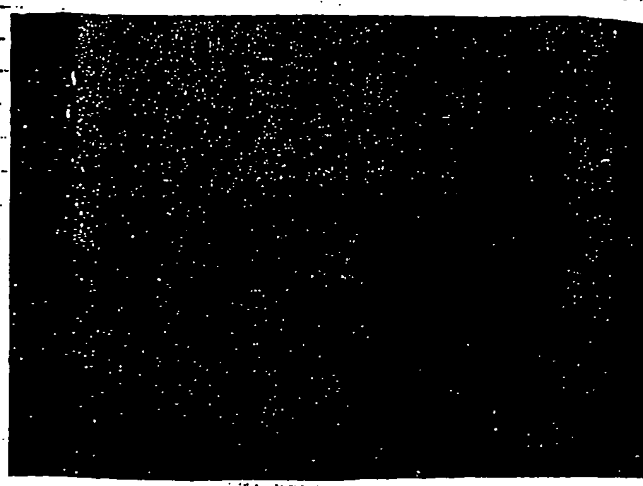
	Kett	10ml [illegible]
#26	[illegible]	2.0
#29	950	1.9
#30	1250	2.4
#34	1150	2.25
#36.	1550	3.0

[illegible] that cultures were induced around 30-50 Klett.
All cultures are [illegible] cells & completely filled with [illegible] of [illegible]
[illegible] cell [illegible] to 30 OD/ml

Western in [illegible] Notebook #32780 p 70

[illegible] to 30 OD & 10 ul per lane.

1. [illegible]
2. 1613 = 50 ng
3. HMS174 pLET4I 37° 5hr
4. 42° 5hr
5. 37° ON
6. 42° ON
7. HMS174 pLET5 37 5hr
8. 42 5hr
9. 37 O/N
10. 42 O/N
11. 101-26
12. 101-29
13. 101-30
14. 101-34
15. 101-36.



[illegible] still look like [illegible] fusions

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DATE

02/26/97 WED 09:01 FAX 908 ...

88

trx-IL13 Coupled Translation Oligos 471/472

pDR107

REVERSE-COMPLEMENT of: U472.oli check: 906 from: 1 to: 50

REVERSE-COMPLEMENT of: U471.oli check: 7686 from: 1 to: 54
trx-il13 coupled translation linker
bsabi-ssti
u471.oli Length: 54 14:45 Type: N Check: 7686 ..

1 GAAGGAGGCT GATTAAATGG GTCCGGTTCC GCCGTCTACC GCTCTGCGTG
R.B.S.
51 AGCT

REVERSE-COMPLEMENT of: U471.oli check: 7686 from: 1 to: 54

u472.oli Length: 50 14:44 Type: N Check: 906 ..

1 CACGCAGAGC GGTAGACGGC GGAACCGGAC CCATTTAATC AGCCTCCTTC

BsaBI

GAT NNNNATC

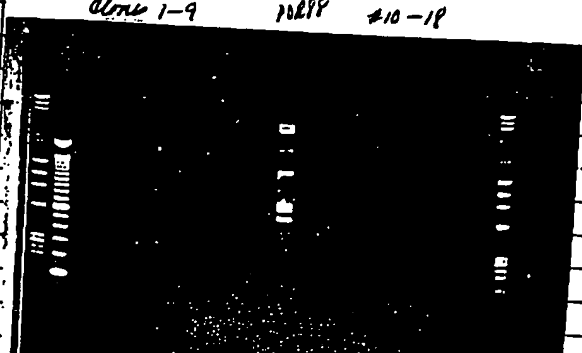
GAT·GCG·AAT·CTG·GCG·TAA
asp ala asn leu ala end
GAT·GCG·AAG·GAG·GCT·GAT·TAA·ATG ....> IL13/SSTI
GAAGGAGG ←— 8bp —→
asp ala lys glu ala asp end met

The coupled system above
1) introduces ribosome binding site GAAGGAGG into the C-terminus of the native thioredoxin protein. Amino acid changes in trx were made as necessary to introduce a strong R.B.S.

BsaBI site 11/17.
BsaBI / BsaHI digest

BsaB I
BsaHI: 2 —759— 2659 —— 3342 —— (6871) — 2
2657 └ 683 ┘ └ 3530 ┘

clones 1-9 pDR98 #10-18



EcoRI PvuII NcoI/BamHI

EcoRI
2920, 2712

PvuII
2350, 2280
1290, 734
108 + 93

Nco/Bam = 917

Different to see & need 7ul DNA.

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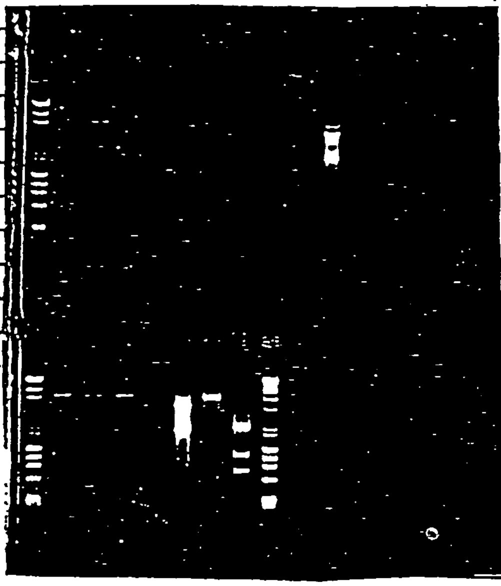
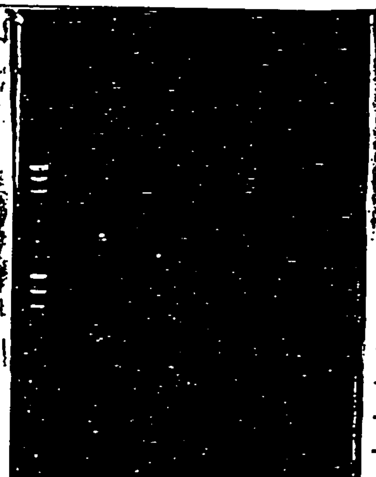


BsBI/BsiE II digest
Should be Anson plasmid
6870 bp.
#2-4-5-10-13-15-16-17-18
look positive.

check SstII site since it is the other cloning site.

SstII/EcoRV Digest.

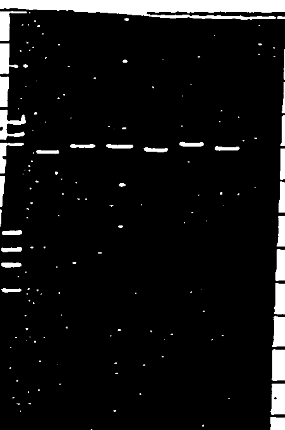


SstII/RV digest
#2-5-13-15-16 maybe 17-18 look positive.

Check fermentations for expression of monomeric ult.
See p. 92-96

Fermentations show 4-5-10-~~13~~-15-16-17-~~18~~ to be positive.

Prepped #13 & #15 for more DNA.



SstI/EcoN1
pshA1/BamH1 } #13
Xba1/BamH1
SstI/EcoN1
pshA1/BamH1 } #15
Xba/BamH1

#13 has no Xba-Bam fragment.

pDF18 EcoN1 @ 1323
SstI @ 834
total 6875 bp
Should be 490 bp fragment.

Note there seems to be an extra EcoN1 site approx 700 bp in #15
The pshA1 site is gone & Xba-Bam frag is the appropriate size.

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Exhibit C

Thio Relaxin - 1L13 fermentation
Time of induction
pDR88 ; pDR99 ; pLET.
Cam Ap Cam/C.
ice chip → flask
slow aeration overnight IFN 8

→ pDR88 | Inoculate @ 30, 90, 200 x.
→ pDR99 | Inoculate @ 30 x
IFN 16R
100ml / 500 ml flask

Grow @ 30°C / 250 rpm a few hours.
Induce w/ 100 uM IPTG and grow @ 15°C / 250 rpm

→ pLET1 | Inoc @ 30x and grow at 15°C / 250 rpm

	Induce @ Klett	18 hrs	43 hrs	67 hrs *
pDR88	30 - 150	770	2300	3000
	90 - 275	1150	2650	3500
	200 - 600	1650	3150	3100
pDR99	30 - 130	700	1900	3150
pLET1	30 x	170	1375	3400

at 43 hrs: Take a 25ml sample + replace cultures at 15°C/250 rpm.
Resusp to 30 od/ml in TEN buffer
insoluble white/green tube
① Sonicate SN → green + yellow labels.
② whole cell GRP → violet labels.
③ Osmotic Shock → purple + magenta
④ Heat denaturation → red + pink.

[Osmotic Shock]
1ml of 30 od/ml.
↓ spin
Resusp 500ul TE (40:5)
add 500ul 40% sucrose.
↓ ice 10 min
spin 30 sec
↓
Resusp in 1ml TE (40:5)
↓ ice 10 min
spin 5 min
Shockate - sup @ 30 od
purple labels
Pellet
↓
GRP to 30 od
magenta la[bels]

[Heat denaturation]
samples, pDR88 / 30x inoculum
pDR99
pLET I.

500ul of unseparated sonicate
heat 0 - 2 - 5 - 10 min
spin 10 min
Sup: SN, red labels
Pellet: GRP to 500ul final, pink labels

* Samples from 67 hrs are labeled as day 2 : Only have SN/insol. sonicated fraction

After 67 Hours : Microscopic examination.
pDR88 = saw a lot of short fat cells w 50% have inclusion bodies
pDR99 = short fat cells, not many inclusion bodies
pLET I = looked like a normal E. coli but it is not producing recombinant proteins. Cells are short thin rods.

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GEL I: Soluble

1	Size std	
2	294 Host	
3	rbuIL-13 std (CHO) 50 ng	
4	pDR88-150	43 Hrs
5	pDR88-275	
6	pDR88-600	
7	pDR99-130	
8	pLET1-30	
9	pDR88-150	67 Hrs
10	pDR88-275	
11	pDR88-600	
12	pDR99-130	
13	pLET1-30	

Gel II:

1	Size Std	
2	pDR88-150	Shocknate (Sol.)
3	pDR88-275	
4	pDR88-600	
5	pDR99-130	
6	pLET1-30	
7	pDR88-150	Shock pellet
8	pDR88-275	
9	pDR88-600	
10	pDR99-130	
11	pLET1-30	
12	pDR88-150	Whole Cell GRP
13	pDR88-275	
14	pDR88-600	
15	pDR99-130	

Gel III: Insoluble Fractions

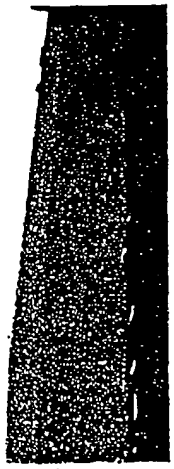
1	Size Std	
2	pDR88-150	43 Hrs
3	pDR88-275	
4	pDR88-600	
5	pDR99-130	
6	pLET1-30	
7	pDR88-150	67 Hrs
8	pDR88-275	
9	pDR88-600	
10	pDR99-130	
11	pLET1-30	
12	pDR88-150	10' Heat Shock Pellets
13	pDR99-130	
14	pLET1	
15	rbuIL-13 Std (CHO) 50 ng	

Gel IV: Soluble Heat Shock Fractions

1	Size Std	
2	pDR88-150	0'
3	pDR88-150	2'
4	pDR88-150	5'
5	pDR88-150	10'
6	pDR99-130	0'
7	pDR99-130	2'
8	pDR99-130	5'
9	pDR99-130	10'
10	pLET1-30	0'
11	pLET1-30	2'
12	pLET1-30	5'
13	pLET1-30	10'
14	IL13 std.	

Extra photos of gels 1-4 in file box

4



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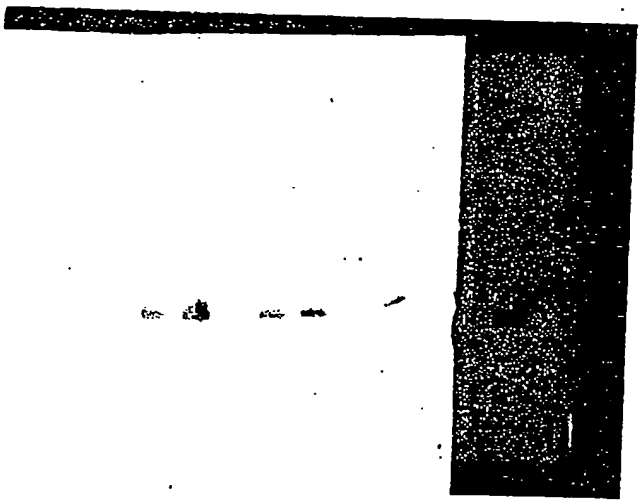
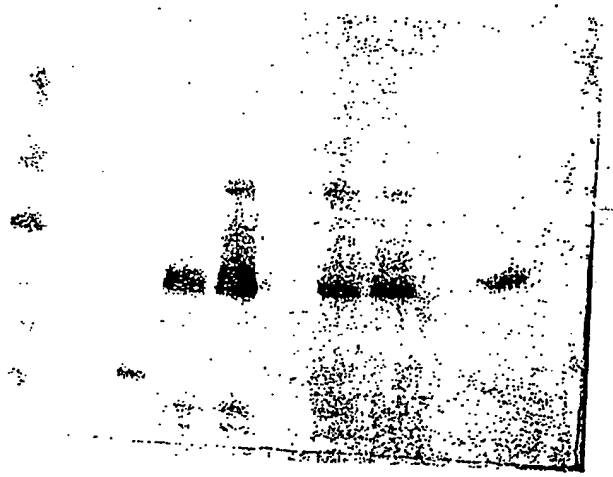


5 & 30 ob before.
2.5 & 15 after dye
Use Kilodaltype
sheet
1113 = 50 ng
pDR88 - 5ul 50D
pDR99 - 5ul 50D
plt1 - 10ul 300D
pDR88 - 5ul 50D
pDR99 - 5ul 50D
plt1 - 10ul 300D
pDR99 - 5ul 50D
5ul after 10min/80°C



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The Reduction - IL13 Coupled Translation
471/472 dejoo pSL 102

Fermentation

Single colony isolated pieces from p. 88 → 25ml IFN IL2 same
Grown @ 30°C 3hrs — Induce w/ 10mM IPTG & upshift to 41°C
Grown ON GRP samples to 30 OD.

#2 - 1800	10 - 775	16 - 750
4 - 950	13 - 1900	17 - 850
5 - 750	15 - 1050	18 - 1950

2 shorter cells w/ polar dark spots
4 extremely long cells - snakes - 2-8 light refractile IBs
5 ~ 50% as 4 & 50% long cells w/ 2 LR IBs per cell
10 same as 4
13 - short cells polar dark spots
15 - long cells typically 1 LR IB per cell
16 - same as 4
17 - same as 4
18 - very short stubby cells ~50% have 1 R IB.

1 dye std + IL13 ckd std
2 #2
(A) 3 #4 positive
4 #5
5 #10
6 #13
(B) 7 #15 positive
8 #16
9 #17
10 #18

4-5-10-15-16-17 negatives

(A) Do have Cotranslation
(B) Do have Accumulation @ 42°C.

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pDR ... Fermentation @ 15°C Test on Solubility Stability Plating 9

... Isolate from ...

Single Colony Isolate again

Single colony → 10ml IFN8 + Cam. Seed growing at 30°C.

↓5ml.

Inoculate 50ml in a 312ml baffled flask. IFN 16 & Cam. Grow at 30°C ~3 hrs.

Add 100 uM IPTG & shift to 15°C.

	Induce at 2:30 Klett	21 hrs	48 hrs	68 hrs	
= 4	45	450	2100	3350	short cells maybe polar dark spots ...
5	55	525	2300	3500	staining ...
10	30	350	1550	3000	Cells ~50% elongated (not snakes) maybe polar dark spots
15	65	350	1400	3000	shortish cells possible dark spots.
16	70	625	2700	3600	Cells are quite short (look a little ...)
17	25	825	3150	3800	Cells are short & otherwise unremarkable.

At 48hrs. Took a 13ml sample & froze pellet for analysis.
Plated #15, #17 for plasmid stability

At 48 hrs no sign of inclusion bodies. Cells were short but longer & thicker than a ... type E. coli.

Stability 48 hrs.

pDR102-15

	+Cam	IFN8		+Cam	IFN8
10^{-8}	37	25	10^{-7}	171	160
	46	57		252	151
	N.D	17		423	311
	83+?	99			

41.5 / 33 = 126% ; 3.3×10^9

136% ; 1.55×10^9

126% / 136%

1400 K = 2.4×10^9

pDR102-17

10^{-8}	92	179
	86	219
	152	145
	330	543

61%

10^{-7} & 10^{-6} plates were too crowded to count.

61%
3150 K = 5×10^{10}

pDR102-15 Colonies were small, glossy, round. Found 2 larger flatter shaped clones. These were SLC as 102-15-101 & 102-15-102

pDR102-17 All colonies were larger, flatter in morphology.

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pDR102

(Extra Photo Are in Drx)

Sol of 5 od.

S-1 pDR102

1	Std	
2	Std 202020	
3	IL13	
4	pDR102-4	48 hr. sol.
5	5	
6	10	
7	15	
8	16	
9	17	
10	pDR102-4	28 hr sol.
11	5	
12	10	
13	15	
14	16	
15	17	

Gel 2 : insoluble

Sol of 30 od

Sol

1	Size	
2	202020 std	
3	IL13 100 ng	
4	pDR102-4	48 hr
5	5	
6	10	
7	15	
8	16	
9	17	
10	pDR102-4	68 hr
11	5	
12	10	
13	15	
14	16	
15	17	

Looks like monomer IL13 & is soluble.

asp ala asn leu ala

TIX 13: GAT·GCT·AAT·CTG·GCG·TAA

asp·ala·Lys·Glu·ala·asp * met

pDR102 GAT·GCG·AAG·GAG·GCT·GAT TAA·ATG·....(IL13) →

R.B.S ← 8 bp → met

TAA

ATG

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